IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Gjerset et al
Title:	TUMOR SUPPRESSION THROUGH BICISTRONIC CO-EXPRESSION OF P53 AND P14ARF
Appl. No.:	10/717,845
Filing Date:	11/19/2003
Examiner:	Priebe, S.D.
Art Unit:	1633
Conf. No.	9478

DECLARATION OF DR. RUTH GJERSET
UNDER 37 C.F.R. § 1.132

I, Ruth Gjerset, Ph.D., state and declare as follows:

1. I am currently Associate Professor for the Sidney Kimmel Cancer Center, San Diego, CA, the assignee of the above-referenced U.S. Patent Application No. 10/717,845 (hereinafter referred to as "the '845 application"). I am named as a co-inventor on the '845 application. I received a Ph.D. in 1977 from the University of California, San Francisco in Biochemistry and Biophysics and have extensive post-doctoral training in Biochemistry and Molecular Biology at the Pasteur Institute in Paris, and at the University of California, San Francisco and San Diego. I have worked in cancer genetics research for 20 years and have published over 39 scientific articles in peer-reviewed journals and review publications. My curriculum vitae is attached. (EXHIBIT A)

2. I have reviewed and am familiar with the specification filed in the '845 application, and the Final Office Action mailed on October 3, 2007, and the non-Final Office Action mailed on February 13, 2007. I have also reviewed and am familiar with Roth et al. (U.S.

Patent 5,747,469), Lu et al. (Cancer Res. 62: 1305-1310, 2002), Tango et al. (Hum. Gene Ther. 13: 1373-1382, 2002), Almond et al. (WO 99/47690), and Teimann (WO 01/11063)..

3. The rejccted claims of the '845 application encompass methods of inducing killing, apoptosis, or growth arrest of malignant p53-positive cancer cells by contacting the cells with a bicistronic construct encoding p53 and p14ARF under the control of a single promoter. The Examiner alleges that these claimed methods are obvious based on the combination of the cited prior art. As I understand the rejection, the Examiner is alleging that Lu et al. and Kim et al. demonstrate that the expression of p53 and p14ARF, from individual vectors (i.e., a dual vector system), is capable of inducing killing, apoptosis, or growth arrest of p53-positive cancer cells. The Examiner further alleges that it would have been obvious to combine the p53 and p14ARF genes into a single vector, under the control of a single promoter, as taught by Almond et al. and Tiemann. As I understand it, this rejection may be overcome by demonstrating that the claimed invention yields surprising or unexpected result.

4. I am familiar with the relevant prior art and have determined that it predicts the use of a single promoter bicistronic construct expressing p53 and p14ARF (both genes encoded on a single vector under the control of a single promoter) would not be significantly better than the use of individual vectors each expressing a single gene (i.e., a dual vector system) for killing p53-positive tumor cells.

5. Kim et al. (Protein Sci., 13: 1698-1703, 2004; EXHIBIT B), and the scientific publications cited therein, disclose that single promoter bicistronic vectors should, in theory, mimic a bacterial operon and effectively express a cluster of genes from a single promoter (p. 1668; Introduction). However, Kim et al., citing Rucker et al., 1997, note the longstanding recognition that the second gene of single promoter bicistronic vectors is underexpressed relative to the first (p. 1668, Introduction).

6. The use of separate vectors each expressing a single gene (i.e., a dual vector system) is an art-recognized alternative (Kim et al. at p. 1668; Introduction). However, dual vector systems may not necessarily be superior (or inferior) to single promoter bicistronic vectors. In some instances, dominance of one vector over the other in the copy number as been reported despite the use of compatible origins of replication (p. 1668, Introduction, citing

Johnston et al., 2000). Thus, the prior art provides no indication that one system would be superior to the other and, in fact, prior art evidence suggests that a single promoter bicistronic system would be relatively comparable to a dual vector system.

7. I have performed experiments comparing the relative effectiveness of the single promoter p53/p14ARF bicistronic vector with a dual vector system. In order to control for possible differences in the level of infection of multiple vectors in the dual vector system compared to the bicistronic (single) vector system, relatively high vector levels were used. This ensured that substantially all of the cells in each system received each vector. As demonstrated by the calculations below, differences in vector efficiency are more pronounced at low multiplicity of infection (moi) levels, but are mitigated as the moi is increased and infection rates approach 100% for all vectors.

8. The fraction of uninfected cells by the two approaches can be quantified by assuming a random distribution of vector particles. The fraction of cells in the population receiving no vector (P_o), is related to the average number of vectors (n) applied per cell (i.e., moi) by the Poisson formula: $P_o = e^{-n}$. Thus, the differences between the two approaches, bicistronic or combination single gene, are more pronounced at low moi.

9. For an moi of 1 for the bicistronic vector or either single vector of the dual vector system:

$P_o = e^{-1} = 0.37$. So 37% receive no vector, and 63% receive both genes.

For the corresponding single gene combination approach (overall moi =2):

$P_o = e^{-2} = 0.14$. So 14% receive no vector, and 86 % receive something.
63% receive p53 (based on the moi=1 calculation above)
63% receive p14 (based on the moi=1 calculation above)
40% (63% x 63%) receive both
46% (86%-40%) receive one or the other.

10. Thus, for an moi of 10 of each vector (the lowest concentration used in the experiments presented below): $P_o = e^{-10} = 4.5 \times 10^{-5}$; meaning that <0.005% of cells receive no

vector, and virtually 100 % receive each individual vector. Furthermore, even at the lowest moi of 10, virtually 100% of the cells in the dual vector system are expected to receive both vectors.

11. In view of these calculations, I performed an experiment using moi from 10-200 for each vector in order to ensure comparable levels of infectivity. This experimental design in conjunction with the prior art suggestion that expression using a bicistronic vector system is approximately comparable to a dual vector system predicts approximately comparable results in the use of the bicistronic p53/p14ARF vector versus a p53 and p14ARF dual vector system.

12. Contrary to the prediction of comparability, the experimental results demonstrate that a single promoter bicistronic vector encoding p53 and p14ARF is far superior to the dual vector system for treating p53-positive cancer cells. In my opinion, this result is both surprising and unexpected.

13. The data provided in EXHIBIT C was generated in my laboratory. These data show experimental results obtained using the p53-positive N2O2 breast cancer cell line following administration of either a bicistronic vector encoding p53 and p14ARF under the control of a single promoter, or simultaneous administration of a p53-encoding vector and a p14ARF-encoding vector. The N2O2 cells were treated essentially as described in Saadatmandi et al., Cancer Gene Ther. 9: 830-839, 2002 (EXHIBIT D). Briefly, N2O2 cells were treated for four hours with the indicated doses of vector and replated in 96-well plates at 3000 cells per well. Each treatment condition was done in triplicate. Cells were incubated at 37°C in 10% CO_2 for three days. Viability was scored using the MTS assay and viability is expressed as a percent of control (no treatment). For cells treated with two vectors, the indicated moi is the moi used for each vector, rendering the total dose of vector twice as much relative to the cells treated with the bicistronic vector. In other words, the cells treated with 50 moi of the bicistronic vector, for example, are compared to cells treated with 50 moi of Adp14 and 50 moi of Adp53 (i.e., 100 moi of total vector). As discussed above, even at 10 moi, it is expected that substantially all of the cells treated with the dual vector system were infected with both vectors using this protocol.

14. As shown in EXHIBIT C, the bicistronic p53/p14ARF vector was significantly more effective at reducing the relative three day growth rate of p53-positive cancer cells compared to treatment with the individual vectors. An moi of 10 for the single promoter

bicistronic p53/p14ARF vector resulted in significantly more growth suppression than 200 moi of each of the p53 and p14ARF individual vectors used in combination. Interpolation of the results suggests that about 5 moi of the bicistronic construct results in approximately the same growth suppression as 200 moi of each individual vector used in a dual vector system. This represents approximately a 40-fold increase in efficacy for the bicistronic vector. This effect is surprising and unexpected in view of the relative comparability predicted by the prior art.

15. In summary, I have discovered that a single promoter bicistronic vector expressing both p53 and p14ARF is significantly more effective for treating p53-positive cancer cells than treatment using a dual vector system in which each of the p53 and p14ARF genes are expressed by individual promoters. This result is particularly surprising and unexpected in view of the prior art suggesting that the effectiveness of a single promoter bicistronic vector comparable to a dual vector system.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both under § 1001 of Capital Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10.31.07
Date

Ruth G. Gjerset
Ruth Gjerset, Ph.D.